THE USE OF PHOSPHORYLATED SUGARS TO SUPPORT PROTEIN SYNTHESIS WITH SOME MAMMALIAN CELL EXTRACTS

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1. Introduction

In vitro protein synthesis by mammalian cell extracts requires a steady state supply of ATP. Therefore, an ATP-generating system is usually added to the extracts [1]. We have previously used creatine phosphate and creatine phosphokinase to generate ATP with good results [2]. However, we have recently noticed that different preparations of creatine phosphokinase support protein synthesis by mammalian cell extracts to a variable extent [3]. With some preparations of this enzyme protein synthesis proceeds linearly for only 15–20 min, whereas with other preparations protein synthesis can proceed linearly for up to 60 min. We subsequently showed that some preparations of creatine phosphokinase contain nuclease activity [3].

This observation encouraged us to investigate alternative methods to generate ATP in cell extracts. A report that phosphorylated sugars added together with purines stimulate protein synthesis in reticulocyte lysates obtained from ATP-depleted cells [4] led us to use these compounds to regenerate ATP in mammalian cell extracts. We find that the addition of some phosphorylated sugars without any enzyme supplement supports greater protein synthesis than creatine phosphate and creatine phosphokinase or other sugars in certain cell extracts.

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2. Methods

HeLa and L cells were grown as in [5]. Ascites cells were obtained from the ascitic fluid of mice injected intraperitoneally with these tumor cells as in [6]. Extracts were prepared from cells washed at 37°C and homogenized as in [2]. Hemin was added to final conc. 50 μM to HeLa cell extracts only. The extracts were stored frozen in liquid N_2. Reticulocytes were obtained from anemic rabbits and lysates were prepared as in [5]. Wheat germ extracts were prepared as in [5].

HeLa, ascites and L cell extracts were incubated as in [5] with an energy-generating system consisting of creatine phosphate and creatine phosphokinase. The composition of incubations containing reticulocyte lysate and wheat germ extract is in [5]. All the incubations contained 50 μM unlabeled amino acids minus lysine, 0.1 mCi/ml [^{3}H]lysine (40 Ci/mmol) and other additions, as indicated in the figure legends. The incubations were carried out at 23°C with the wheat germ extract and at 30°C with the animal cell extracts. In order to test whether sugars would support protein synthesis, the composition of the incubation mixtures was modified by omitting creatine phosphate and creatine phosphokinase and changing the composition of other components. These incubations contain in final vol. 50 μl: 35 μl cell extract; 10 μl 5 mM ATP; 1 mM GTP; 100 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid/KOH buffer, pH 7.5; 50 μM unlabeled amino acids minus lysine; 5 μCi [^{3}H]lysine; 5 μl sugar solutions to give the final sugar concentra-

tion indicated in the figure legends. Reactions were supplemented with 120 mM K(OAc) and 2 mM $Mg(OAc)_2$ by desiccating these salts in the assay tubes. Aliquots, 5 μl, of the incubations were processed for counting as in [2].

3. Results

Protein synthesis was measured in 60 min incubations in the presence of different concentrations of glucose, glucose 6-phosphate and fructose 1,6-bisphosphate (Fru-P_2). The activity of these compounds in supporting protein synthesis was determined in 5 cell extracts. In all extracts, glucose had relatively little effect on protein synthesis (fig.1 and data not shown). In ascites, HeLa and L cell extracts, glucose 6-phosphate stimulated protein synthesis at 2–4 mM concentration but was somewhat inhibitory at higher concentrations. Fru-P_2 supported the most protein synthesis with little or no inhibition at high concentrations (fig.1). None of these compounds supported protein synthesis in reticulocyte lysates translating endogenous mRNA or in the wheat germ cell free system translating added globin mRNA. As controls we used incubations with added creatine phosphokinase energy-generating system or with added ATP and GTP alone. The amino acid incorporation observed with added sugars in the wheat germ and reticulocyte cell-free system was approximately equal to that obtained with addition of ATP and GTP alone, which was about 5% of that obtained with added creatine phosphokinase and creatine phosphate (data not shown).

The time course of protein synthesis was determined in an ascites cell extract supplemented with 4 mM Fru-P_2, or with creatine phosphokinase and creatine phosphate, or with ATP and GTP alone (fig.2A). With Fru-P_2 protein synthesis proceeded for 90 min (the last point tested), whereas it stopped after 15 min with creatine phosphokinase or ATP/GTP.

When both Fru-P_2 and creatine phosphokinase energy-generating system were added together, protein synthesis proceeded at the same rate as in the incubation with Fru-P_2 alone (data not shown). This indicated that the stimulation of protein synthesis in incubations with Fru-P_2 relative to incubations with

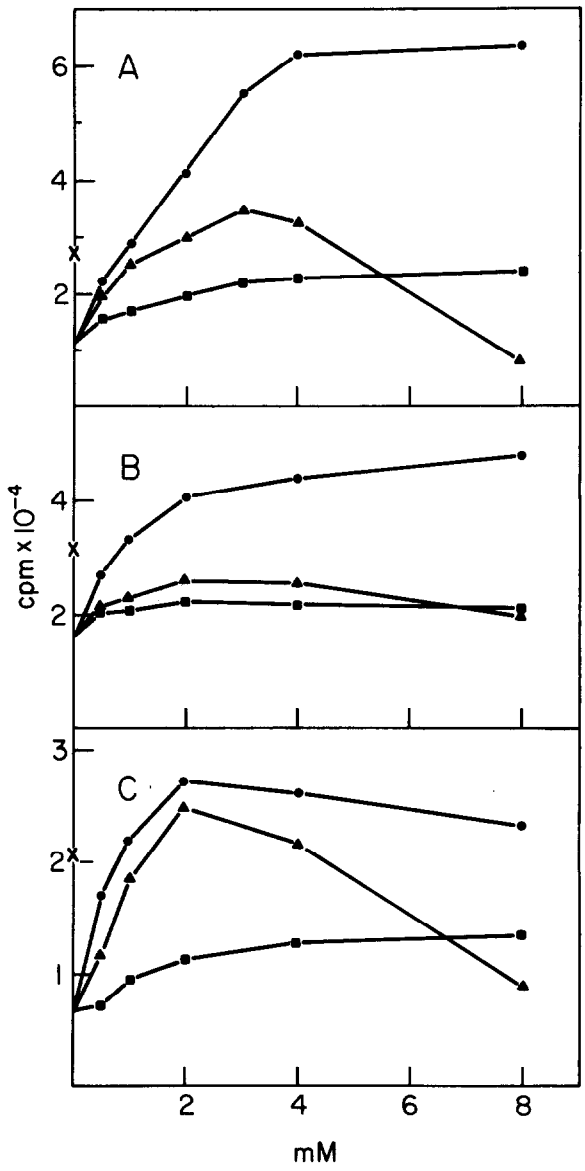

Fig.1. Stimulation of protein synthesis by glucose (■–■–■), glucose 6-phosphate (▲–▲–▲) and fructose 1,6-bisphosphate (●–●–●) in extracts from ascites (A), HeLa (B) and L cells (C). The incubations were assembled as in section 2 and contained the final concentrations of sugars indicated in the abscissa. Cold lysine, 20 μM, was added to the ascites cells extract. The incubations were for 60 min at 30°C and 5 μl aliquots were taken for counting. On the ordinate are indicated the incorporation obtained with no added sugars (at the intercept of the curves) and the incorporation obtained with the creatine phosphate/creatine phosphokinase energy-generating system (X).

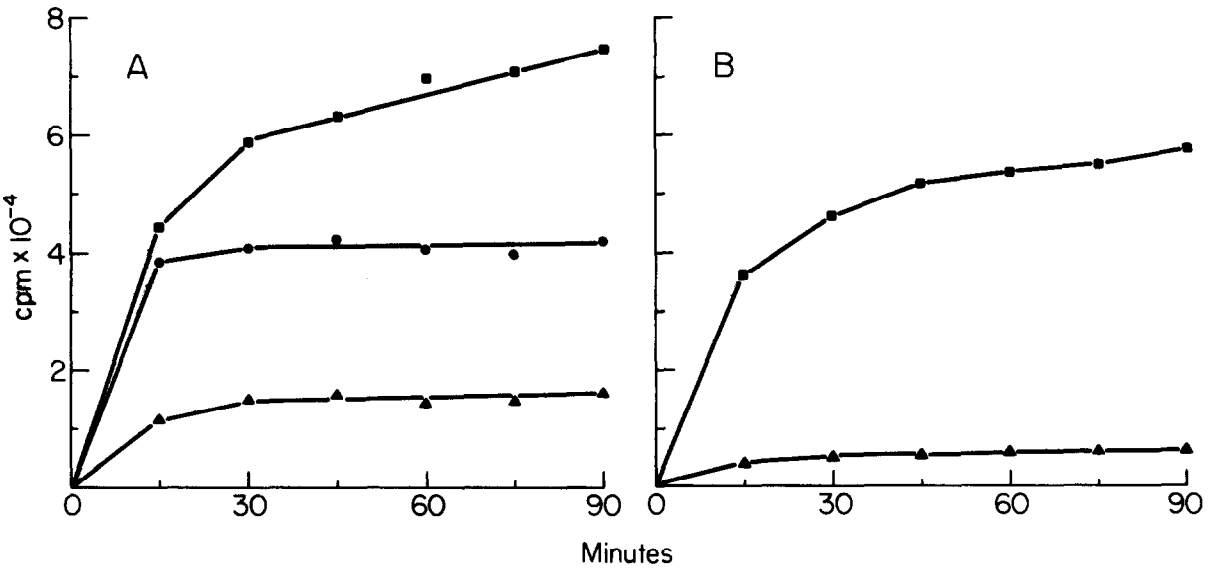

Fig.2. Time course of protein synthesis in an ascites extract supplemented with 4 mM Fru-P_2, 0.2 mM GTP and 1 mM ATP (A) or 1 mM ADP (B) (■–■–■); with creatine phosphate, creatine phosphokinase, 0.2 mM ATP and 0.2 mM GTP (●–●–●); with 0.2 mM GTP and 1 mM ATP (A) or 1 mM ADP (B) (▲–▲–▲). The incubations were assembled as in fig.1 legend with 20 μM cold lysine added. At the times indicated in the abscissa 5 μl aliquots were taken for counting.

creatine phosphokinase alone was not due to the presence of nuclease activity in the particular preparation of this enzyme used. The stimulation of protein synthesis by phosphorylated sugars was determined in extracts of different types of cells in tissue culture and found to be quite variable (data not shown). However, in most cases the incorporation obtained with an optimal concentration of Fru-P_2 was found to be higher than that obtained with the creatine phosphokinase energy-generating system. With some extracts Fru-P_2 and creatine phosphokinase were equally effective in supporting protein synthesis. Evidence that Fru-P_2 generated ATP was obtained by substituting ADP for the ATP added to cell extracts. About the same amount of protein was synthesized with added ADP as with added ATP (fig.2B).

4. Discussion

Phosphorylated sugars can generate ATP and support protein synthesis in some cell extracts. The use of phosphorylated sugars may become the method of choice in studies on mRNA stability during in vitro protein synthesis, since no addition of enzyme to the cell extracts is necessary, and consequently the risk of adding nuclease activity during the assembly of the incubations is minimized. Another important advantage is the generally greater stimulation of protein synthesis obtained with Fru-P_2 relative to that obtained with preparations of creatine phosphokinase. Fru-P_2 and possibly other phosphorylated sugars have a stimulatory effect on protein synthesis, which cannot be accounted for simply by their ability to generate ATP. In incubations with the creatine phosphokinase energy-generating system, phosphorylated sugars like glucose 6-phosphate, fructose 6-phosphate and Fru-P_2 promote binding of Met-$tRNA_f$ to native 40 S ribosomal subunits [8]. A similar effect in incubations where phosphorylated sugars only are used to support protein synthesis might explain the stimulation observed.

Only extracts of tumor cells (HeLa and ascites) or of cells adapted to continuous growth in tissue culture (L cells) seem capable of utilizing phosphorylated sugars to support protein synthesis. The reticulocyte and wheat germ extracts, which are among the most active extracts for in vitro protein synthesis, cannot

use the sugars tested to support protein synthesis. This may reflect the different way in which different cells generate ATP. For example, in cells transformed by oncogenic viruses, there is an increased glycolytic flux relative to normal cells [9]. The level of phosphorylated sugars present in different cells is consequently quite different. Fru-P_2 is present at 60 μM in rat skeletal muscle [10,11], but at 10 mM in ascites cells [12]. In addition, the utilization of phosphorylated sugars by different cell extracts may be controlled in different ways by the complex regulatory mechanisms of carbohydrate metabolism. However, we do not as yet have an explanation for the failure of Fru-P_2 to support protein synthesis in reticulocyte lysate and wheat germ extract. We believe that in extracts of tumor cells or of cells adapted to continuous growth in tissue culture Fru-P_2 is used to generate ATP, since ADP can be substituted for ATP in the incubations without significant decrease in the activity of the extracts. Our observations provide a simple and effective procedure for carrying out protein synthesis with extracts of these cells.

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